AFRL-RH-WP-TP-2015-0012

Host-Pathogen Coupled Interactions

Peter J. Robinson
C. Eric Hack
Jeffery M. Gearhart

Bioeffects Division Molecular
Bioeffects Branch

Henry M. Jackson Foundation
for the Advancement of Military Medicine
Wright-Patterson AFB OH

Jan 2015

Interim Report for October 2012 - October 2014

Air Force Research Laboratory
711th Human Performance Wing
Human Effectiveness Directorate
Bioeffects Division
Molecular Bioeffects Branch
Wright-Patterson AFB OH 45433-5707

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation; or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

The experiments reported were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

(AFRL-RH-WP-TP - 2015 - 0012) has been reviewed and is approved for publication in accordance with assigned distribution statement.

YU.KYUNG.OH.1228570016

Digitally signed by YU.KYUNG.OH.1228570016
DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USAF, cn=YU.KYUNG.OH.1228570016
Date: 2015.04.02 11:12:08 -04'00'

KYUNG YU, Work Unit Manager
Molecular Bioeffects Branch

POLHAMUS.GARRETT.D.1175839484

Digitally signed by POLHAMUS.GARRETT.D.1175839484
DN: c=US, o=U.S. Government, ou=DoD, ou=PKI, ou=USAF, cn=POLHAMUS.GARRETT.D.1175839484
Date: 2015.04.02 11:37:32 -05'00'

GARRETT D. POLHAMUS, DR-IV, DAF
Chief, Bioeffects Division
Human Effectiveness Directorate
711th Human Performance Wing
Air Force Research Laboratory

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government’s approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
04-01-2015	Interim	Oct 2012 – Oct. 2014

4. TITLE AND SUBTITLE
Host-Pathogen Coupled Interactions

5a. CONTRACT NUMBER
NA

5b. GRANT NUMBER
NA

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Robinson, Peter J.[1], Hack, C. Eric[1], Gearhart, Jeffery M.[1]

5d. PROJECT NUMBER
ODTW

5e. TASK NUMBER
P0

5f. WORK UNIT NUMBER
ODTWP005/ H069

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
[1]HJF, 2729 R St, Bldg 837, Wright-Patterson AFB OH 45433-5707

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Materiel Command*
Air Force Research Laboratory
711th Human Performance Wing
Human Effectiveness Directorate
Bioeffects Division
Molecular Bioeffects Branch
Wright-Patterson AFB OH 45433-5707

10. SPONSOR/MONITOR'S ACRONYM(S)
711 HPW/RHDJ

11. SPONSORING/MONITORING AGENCY REPORT NUMBER
AFRL-RH-WP-TP-2015-0012

12. DISTRIBUTION AVAILABILITY STATEMENT
Distribution A: Approved for public release (PA); distribution unlimited. PA Case No: 88ABW-2015-1836 Date Cleared: 9 April 2015

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Understanding the complex interactions of pathogens and host macrophages during the initial stage of infection (generally the first 12-48 hours) will help identify key targets for potential therapeutic interventions and provide information to optimize both drug treatment timing and dosing characteristics. As a pathogen moves from the external environment into the host, the pathogen is endocytosed by host immune cells, and in the course of infection can escape back into the tissue or bloodstream of the host. During this process the pathogen responds to time-varying environmental triggers by activating and modulating its own signaling pathways so as to produce appropriate responses that facilitate its survival. Many of these responses modulate host pathways and thereby alter the pathogen's environment. In this way multiple interacting feedback loops are set up between the pathogen and its host. The current proposal aims to understand these interacting pathways and develop quantitative predictive models of both host cell and pathogen behavior during the initial course of intracellular pathogen infection, so as to elucidate signaling mechanisms elicited and within *Francisella tularensis* (FT), *Yersinia pestis* (YP) and others (*Burkholderia mallei* and/or *B. pseudomallei)* during infection, particularly in response to specific environmental conditions occurring within host compartments, as well as interactions between these signaling networks in bacteria and those within host responder cells (e.g., macrophages), and their combined effect on the host's overall (immune) response.

15. SUBJECT TERMS
Mathematical model, signaling pathways, bacterial infection, macrophage, immune system

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U					K Yu
a. REPORT	b. ABSTRACT	c. THIS PAGE	SAR	5	19b. TELEPONE NUMBER (*Include area code)*
U	U	U			NA

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI-Std Z39-18

Annual Research Summary from October 1 2012 to September 30 2014

Host-Pathogen Coupled Interactions

Work Unit Monitor: Kyung O. Yu, Molecular Bioeffects Branch, Bioeffects Division, Human Effectiveness Directorate, 711 Human Performance Wing/RHDJ, Air Force Research Laboratory

WorkUnit#: ODTWP005/ H069

This is a 3 year project supported by DTRA, and is a collaborative effort with the University of Houston and UCLA. The study research is expected to finish in FY15.

Objective: To elucidate mutually adaptive host-bacterial pathways and model the associated signaling mechanisms within *Francisella tularensis* (FT), *Yersinia pestis* (YP) and others (*Burkholderia mallei* and/or *B. pseudomallei)* during mammalian host infection, particularly in response to specific environmental conditions within host compartments, as well as interactions between these signaling networks in bacteria and those within host early responder cells (e.g., macrophages), and their combined effect on the host's overall response with focus on immune system participation.

Technical Approach: Understanding the complex interactions of pathogens and host macrophages during the initial stage of infection (generally the first 12-48 hours) will help identify key targets for potential therapeutic interventions and provide information to optimize treatment in terms of both its timing and dosing characteristics. As a pathogen moves from the external environment into the host, the pathogen (either spore or live agent) is endocytosed by host immune cells (generally macrophages), and in the course of infection can escape back into the tissue or bloodstream of the host. During this process the live pathogen responds to time-varying environmental triggers by activating and modulating its own signaling pathways, and eliciting appropriate host responses that facilitate its own survival. In this way multiple interacting feedback loops are set up between the pathogen and its host. The current project aims to understand these interacting pathways and develop quantitative predictive models of both host cell and pathogen behavior during the initial course of infection.

Progress:

Mitogen Activated Protein Kinase (MAPK) and Nuclear Factor-kappa B (NF-kB) Signaling Pathways in Y. pestis and B. anthracis

Bacterial virulence is in part dependent on the subversion of host cell defense mechanisms by specific effector molecules produced by the bacteria, often mediated by specific secretion systems. Such effector molecules accumulate in specific compartments, such as the cytosol of immune cells, and interact with cellular defense mechanisms or cell maintenance machinery (see Figure 1). An analysis of the kinetic and dynamic behavior of these effector molecules can provide some insight into bacterial virulence. For example, *Yersinia pestis* secretes a number of Yersinia outer proteins (Yops) via a type 3 secretion system, and one of these (YopJ) accumulates in the cytosol of macrophages where it interferes with the MAP kinase pathway in the host, via acetylation of the MAPK kinase (MAPKK) intermediate, leading to macrophage cell death. Similarly, *Bacillus anthracis* (BA) produces lethal factor (LF) that also accumulates in the cytosol of macrophages, cleaving the MAPKKs and leading to macrophage death. The macrophage MAPK pathway signaling produces genomic transcription and cytokine expressed protein products that are emitted from the infected cell to alert the entire host immune system of an infectious agent. To model these intracellular events, we have developed an *in silico*

quantitative model of cytosolic LF attacking the host cell's MAPK signaling pathway that includes the time-course of LF accumulation in the cytosol, and LF-mediated cleavage of MAPK kinases in terms of a second order rate constant. Applied to *in vitro* data (Muehlbauer et al., *Cell Cycle* (2007) vol. 6 (6) pp. 758-66; Pellizzari et al, *FEBS Lett* (1999) vol. 462 (1-2) pp. 199-204), sensitivity analysis shows that LF half-life is critical to the sensitivity of AKR, BL/6, DBA and human macrophages to LF (with their viability half-lives of 48-72 hours *in vitro*), but this parameter is not important for the survival of RAW264.7, J774A.1 or BALB/C macrophages. The latter have shorter half-lives of 1-3 hours, and macrophage viability is primarily determined by the initial LF influx into the cytosol. By mechanistically describing LF- (and YopJ-) dependent macrophage viability, bacterial death and production of cytokines that recruit additional immune cells and modulate the immune response, the models form a link between organism-level models of infection that describe bacterial proliferation in the host (and the host's immune response), and molecular-level models describing the subversion of the molecular machinery of the immune cells themselves.

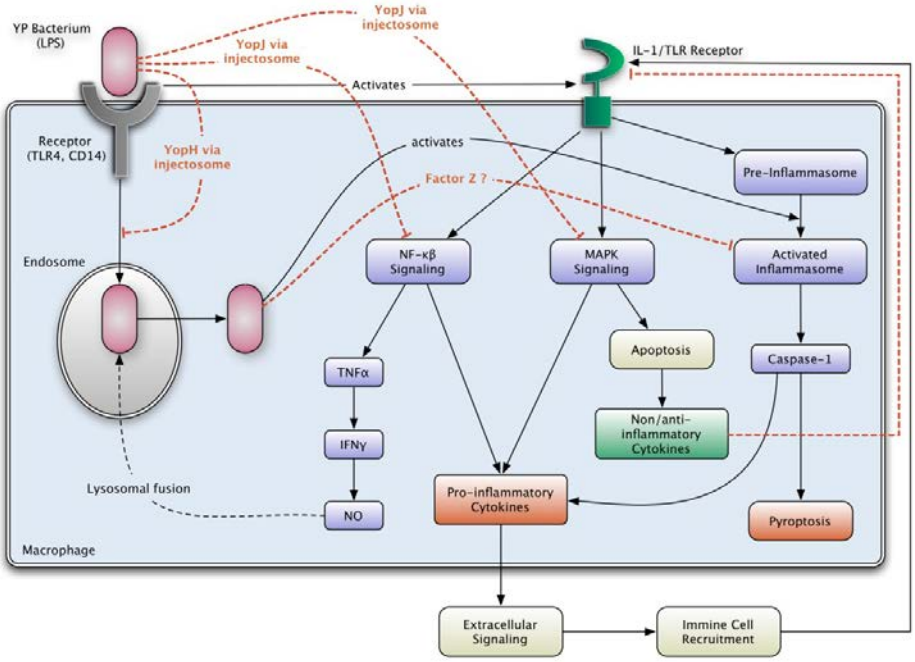

Figure 1. *General interactions of the gram negative bacteria Yersinia pestis (YP) with signaling pathways in host macrophage.*

Multi-Nucleated Giant Cell (MNGC) Formation in Burkholderia

Proliferation of *Burkholderia* bacteria is critically dependent on the formation of multi-nucleated giant cells (MNGCs) in which fusion of host cells is induced by the bacteria (via its type 6 secretion systems, T6SSs). Bacteria proliferate within these giant cells, which ultimately die, resulting in the formation of plaques characteristic of *Burkholderia* infection. The mechanisms of MNGC formation and bacterial proliferation are being explored experimentally in the Miller lab (UCLA), leading to the development of specific mechanistic hypotheses (French, et al. , *Proc.*

Nat. Acad. Sci. 108, 12095–12100, 2011). To date we have modeled MNGC formation using ordinary differential equations (ODEs) to describe bacterial proliferation determined by substrate depletion, together with a probabilistic estimation of membrane fusion between adjacent macrophages. We are also developing agent-based models (ABMs) of this process in NetLogo™. Advantages of ABMs include the ability to take into account spatial resolution and behavior, specific geometries and shapes, movement of individual entities such as bacteria and macrophages, and stochasticity (ability to model very small numbers of entities). We are currently developing an ABM that describes bacterial movement in a fixed macrophage field. The bacteria themselves are modeled as agents, moving within a static field consisting of the interior of macrophages, and the initial conditions can be set up to emulate specific configurations (geometries) of macrophages. We are extending this model to include substrate depletion, where the substrate is assumed evenly distributed within the macrophage. This modification involves implementation of ODEs (in this case application of the Monod equation) within the macrophages that determines the proliferation of the bacteria, and ultimately the probability of macrophage cell fusion. This work was presented (Hack) at the 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC'14), August 26-30, 2014, Chicago, IL.

Modeling of pathogen response to host-modulation of iron

F. tularensis (Ft) requires iron for survival and host cells are believed to possess relatively low amounts of free iron and can potentially modulate iron availability in response to infection. We are developing *in silico* and *in vitro* models to study the impact of iron modulation on pathogen survival. Using the replicated *E. coli* iron acquisition model by Semsey, et al. (*Nucleic Acids Research*, 34, 4960–4967. 2006) as an initial model for iron acquisition, we optimized the model based on Ft iron acquisition data from published empirical studies. This work is part of a collaborative effort with the University of Houston.

Modeling host reactive oxygen species and nitric oxide pathways

We have completed the model for the pathways involved in host-generation of reactive oxygen species and reactive nitrogen intermediates (ROS/RNI) stresses that impact the growth of all intracellular pathogens. To model the complexity of the macrophage intracellular response to bacterial lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) stimulation, and their effect on inducible-nitric oxide synthase (iNOS) gene expression, we developed a kinetic model that includes a comprehensive representation of intermediate signaling molecules, positive and negative feedback inhibitory proteins that modulate the production of iNOS, as well as the metabolic pathway for NOS production. Although there exist previously published reports on individual kinetic models for both the JakStat and MAPK pathways, we have formulated a model that takes into account the synergistic role of both pathways in the induction of iNOS. This work is part of a collaborative effort with the University of Houston.